Review Article

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The choice of progestogen for HRT in menopausal women: breast cancer risk is a major issue

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Abstract:
Doctors and patients fear the risk of breast cancer when using hormone replacement therapy (HRT). This review focuses on the choice of progestogen for HRT in menopausal. The Women's Health Initiative (WHI) has been the only large double-blind placebo-controlled study testing the risk of breast cancer (BC) using HRT. No increased risk using estrogen (E)-only was seen, there was a significant decrease in mortality due to BC after the use of HRT which persisted during the recent 18-year follow-up of the WHI. In contrast in the combined arm the risk increased. In about 20 observational studies using mostly medroxyprogesterone acetate (MPA) or estradiol-norethisterone acetate (NETA) an increased BC-risk was observed comparable with the WHI. Only for natural progestogen, progesterone and for dydrogesterone (retro-isomer of progesterone) was no increased risk seen for up to 5–8 years, when compared directly with other progestogens, but for longer treatment an increased risk cannot be excluded. In contrast, the mortality due to BC after use of E-only and combined HRT decreased in about a dozen observational studies, and was very recently confirmed in a Finnish study evaluating 490,000 women using estradiol (E2) plus different progestogens. There have been already more than 70 studies evaluating the risk of BC during HRT, and still there are many open questions. Therefore, this review covers our own and other experimental research which could answer important questions. Experimental research has demonstrated that certain synthetic progestogens, but not progesterone and to some extent also not dydrogesterone, can accelerate the proliferation of breast cancer cells in vitro and in animal studies via special cell membrane components which we recently also detected in patients with BC, and we found differences comparing all available synthetic progestogens. Derived from these mechanisms future research may provide screening for patients at risk and predict the prognosis of possible BC.

Keywords: breast cancer, experimental and clinical research, hormone replacement therapy (HRT), progestogens, Women's Health Initiative (WHI)

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Introduction

The role of progestogen in addition to estrogen (E) therapy in postmenopausal women has come under investigation since the results of the E-only arm of the study Women's Health Initiative (WHI) as compared to the WHI combined arm were published [1], [2]. The most important study WHI was stopped in 2002 due to increased risk of venous thromboembolism, myocardial infarction, stroke and breast cancer (BC) [1]. Stopping the E-only arm [2] was not reasonable but it was largely a political decision. The WHI was performed in two separate trials, in non-hysterectomized women (n = 16,608) using conjugated equine estrogens (CEEs), 0.625 mg/day continuously combined with medroxyprogesterone acetate (MPA) 2.5 mg/day [1], and in hysterectomized women (n = 10,739) using CEE-only (0.625 mg/day) [2] (study duration was 5 and 7 years, respectively). This study has been the basis of most official recommendations, e.g. the guidelines of the International Menopause Society (IMS) [3], the European Menopause and Andropause Society (EMAS) [4], the North American Menopause Society (NAMS) [5], the German Menopause Society [6], the American Endocrine Society [7], the NICE guideline [8],

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the global consensus of 10 societies [9] or very recently the US Preventive Services Task Force [10]. The main results are summarized in Table 1.

Table 1: Women's health initiative (WHI): most important results – hazard ratio (HR) and absolute "Excessive Risk" in both study arms[a].

Clinical Endpoint	Combined Estrogen/Progestin Study Arm				Estrogen–Only Study Arm			
	Placebo[b]	CEE/MPA[b]	HR (95% CI)	ExcR	Placebo[b]	CEE[b]	HR (95% CI)	ExcR
Breast cancer	30	38	1.24 (1.01–1.54)	+4	33	26	0.77 (0.59–1.01)	–3.5
CHD	33	39	1.24 (1.00–1.54)	+3	54	49	0.91 (0.75–1.12)	–2.5
Stroke	21	29	1.41 (1.07–1.85)	+4	32	44	1.39 (1.10–1.77)	+6
VTE	16	34	2.11 (1.58–2.82)	+9	21	28	1.33 (0.99–1.79)	+3.5
Dementia[c]	22	45	2.05 (1.21–3.48)	+11.5	25	37	1.49 (0.83–2.66)	+6
Colon cancer	16	10	0.63 (0.43–0.92)	–3	16	17	1.08 (0.75–1.55)	+0.5
Endometrial cancer	6.9	5.6	0.81 (0.48–1.36)	–0.7	–	–	–	–
Ovarian cancer	2.7	4.2	1.58 (0.77–3.24)	+0.8	–	–	–	–
Hip Fracture	16	11	0.67 (0.47–0.96)	–2.5	17	11	0.61 (0.41–0.91)	–3
Fractures total	199	152	0.76 (0.69–0.83)	–23.5	195	139	0.70 (0.63–0.79)	–28
All Mortality	53	52	0.98 (0.82–1.18)	–0.5	78	81	1.04 (0.88–1.22)	1.5

[a]Mean age 63 years, range 50–79 years. [b]Number of patients per 10,000 women per year. [c]Subgroup age > 65 years (Mean 70 years), perhaps dementia of mixed (vascular type) (increase of M. Alzheimer risk not significant). Modified according Kenemans P. *Maturitas* 2005;51:1–3. ExcR, excessive risk/1000 women per 5 years; CEE, Conjugated equine estrogens 0.625 mg/day; MPA, Medroxyprogesterone acetate 2.5 mg/day; HR, Hazard ration; CI, Confidence interval; CHD, Coronary heart disease; VTE, Venous thromboembolism.

In contrast to the WHI combined arm, in the E-only arm, performed in hysterectomized women, no increase but rather a reduction of BC risk was found, which was significant for patients with more than 80% adherence to study medication according to a sensitivity analysis which was published latre [11]. This result indicates a negative effect of progestogens concerning BC risk. In addition, with E-only there was no increased risk of coronary heart disease (CHD) in contrast to the combined arm, which is important, because the primary aim of the study was to test if hormone replacement therapy (HRT) can be used to prevent CHD. Hot flushes, the leading symptom of climacteric women and most important indication of HRT, was present in WHI only in less than 7% of the women. Although in absolute numbers these risks, mainly derived from the progestogen component, are low, about 0.1–0.5%/year, the decision for or against using HRT for treating an individual patient will concentrate on these main risks in HRT – especially on effects in the breast and in the cardiovascular system.

Whereas the increased cardiovascular risks can be reduced using transdermal instead of oral E, like venous thromboembolism [12], [13], [14], [15] and stroke [16], [17], [18], especially using progesterone as the progestogen component (reviews e.g. [19], [20]), the BC risk seems to be very complex as has already been reviewed extensively in this journal [21], [22]. As this remains the main issue in the context of the use of HRT in postmenopausal women, this review summarizes the present knowledge especially in context with new results of follow-up evaluation and assessment of the WHI and experimental research on the action and mechanisms of progestogens acting on breast cell proliferation. Further clinical studies on the risk of BC, the mortality due to BC, during or after the use of HRT, respectively, is summarized with a special perspective on the progestogen component.

New and recent assessment of the WHI's results

A new release of data from the WHI study has concluded that 'among postmenopausal women, hormone therapy with CEE plus MPA for a median of 5.6 years or with CEE alone for a median of 7.2 years was not associated with risk of all-cause, cardiovascular, or cancer mortality during a cumulative follow-up of 18 years' [23]. For the CEE + MPA trial, the median post-intervention follow-up was 12.5 years and the cumulative follow-up was 18 years; for the CEE-alone trial, the median post-intervention follow-up was 10.8 years and the cumulative follow-up was 18 years. During the intervention phase, all-cause mortality in the pooled cohort was 4.0% with hormone therapy vs. 4.0% with placebo. Compared with placebo, women randomized to receive CEE + MPA had a hazard ratio (HR) of 0.97 [95% confidence interval (CI) 0.82–1.16] and women randomized to receive CEE alone had a HR of 1.04 (95% CI 0.89–1.22). During the post-intervention period, the HR for all-cause mortality was 1.04 (95% CI 0.97–1.10) for CEE + MPA and 0.92 (95% CI 0.85–0.99) for CEE alone. Comparison of women aged 50–59 years with those aged 70–79 years showed that the risk was significantly lower for the younger age

group, as ratios of nominal HRs for all-cause mortality in the pooled cohort were 0.61 (95% CI 0.43–0.87) during the intervention phase and 0.87 (95% CI 0.76–1.00) during the cumulative 18-year follow-up.

Based on these follow-up results of the WHI it was suggested that the safety concerns that were attributed in the past to HRT need to be reconsidered and to be put in their right perspective. In addition, two principle investigators of the WHI recently published that the results assessed in the total WHI-population should not be extrapolated to the benefits and risks of HRT if started early, as in the subgroup of the WHI between 50 and 59 years [24]. Both authors regret the wrong interpretation of the WHI for years and that young doctors do not know how to decide to perscribe HRT and for differentiated individual prescriptions. Another author very recently described the almost unbelievable conditions for the first publication of the WHI [25], pointing out that the paper was written by the Project Officer, and submitted to the journal *JAMA* without the involvement of all the main investigators who were listed as co-authors. These investigators wanted to make changes – but *JAMA* indicated the issue was already printed and ready to be mailed and the press and media have been already informed about the premature stop of the WHI. "*The initial paper was written by a small group from the coordinating center and program office and submitted to the journal without informing or consulting the clinical site principal investigators..... The investigators most capable of correcting the critical misinterpretations were actively excluded from the writing and dissemination activities.*" (Langer [25]).

Despite those problems in the WHI study, this trial with its two study arms is the only large randomized placebo-controlled study which is available to assess the effect of a progestogen added to E in terms of clinical endpoints. Obviously, the main difference between E-only therapy (used in hysterectomized women) compared to E/progestogen, i.e. combined HRT, is related to the risk of BC although in the 18-year cumulative follow-up [23] the increase of BC risk in the combined arm was not significant (HR 1.44; 95% CI 0.97–2.15), but was clearly different to the significant decrease of BC in the CEE-only arm (HR 0.55; 95% CI 0.33–0.92). Thus, the difference in terms of the risk of BC shown during the interventional phase of the WHI persisted during the long-term follow-up of more than 12 years. In contrast, the increased risk of CHD during combined HRT was no longer seen in the 18-year cumulative follow-up (HR 1.05; 95% CI 0.89–1.23) although a trend of lower risk using E-only can be suggested (HR 0.89; 95% CI 0.75–1.05).

The main problem of the WHI is, that this study cannot reflect practical conditions: In the WHI only one HRT preparation was tested, and only one dosage was used which is, as we know today, the two-fold of the dosage which we would use in women with a mean age about 65–67 years – the mean age during the study. For the most part in Europe we mostly use various other forms of therapies regarding type of hormones, dosage and application form. On average the women were too old at start with HRT (about 65 years of age), and about 50% of the population were at high risk particularly for BC and cardiovascular diseases. In addition, as about 40% were obese women [body mass index (BMI) > 30 kg/m^2], it is questionable if any HRT may have had relevant preventive or therapeutic effects because most of those women already produced endogenous estradiol from large depots of estrone sulfate. Rather the addition of exogenous Es may lead to overdosing as, for example, can be suggested by the fact that only those obese women have demonstrated to be at an increased risk of BC in the WHI.

Thus, even though we must always consider the results of the WHI, we have to add the results of observational studies. We must also investigate the biological plausibility for increased BC risk induced by different progestogens, like the proliferation effects of breast tissue in vitro and in vivo, also evaluating the possible risk factors and the mechanisms of the development of the disease. In the following the effect of progestogens added to E is summarized according to extensive own and other experimental research, especially comparing the use of natural progestogen, progesterone, with the effect of synthetic progestogens.

Experimental BC research: genomic and non-genomic actions

Two receptors have been identified to be mainly responsible for the physiological and biological effects of progestogens, i.e. progesterone receptor A (PR-A) and B (PR-B). Like the E receptors, both (PR-A and PR-B) are capable of ligand binding, of dimerization and interaction with responsive DNA elements in the promoter region of target genes and of modulating the transcriptional machinery which catalyzes gene expression. Ligand-bound PRs dimerize to form homo- or heterodimers resulting in three possible combinations: A:A, A:B or B:B. PR-A and PR-B may have various transcriptional activities and functions in normal mammary gland development [26], [27]. This is suggested from studies of PR-A or PR-B gene-deleted mice and from PR-A or PR-B overexpressing transgenic mice [27], [28].

Numerous experimental studies have shown that synthetic progestogens bind to human PR, most of them displaying a higher relative binding affinity than progesterone itself (e.g. [29], [30], [31]). However, binding properties differ depending on the experimental conditions and therefore results on receptor binding affinities should be handled cautiously. In addition, some data do not clearly indicate whether the binding affinities were measured with PR-A or PR-B. It is conceivable that the synthetic progestogens differ in their binding properties

to PR-A or PR-B. However, most tested progestogens were similar in their activity to trigger qualitatively similar expression profiles of endogenous PR-regulated genes in human BC cell lines [32], [33]. Thus, for genomic actions it has been expected that most of the effects of progestogens, of progesterone and synthetic progestogens, are group effects with differences in vivo only regarding different pharmacokinetic but not regarding pharmacodynamic action.

However, in addition to PR-A and PR-B further nuclear receptors might be involved in the action of progestogens on the breast. Most progestogens present partial binding affinities to other steroid receptors like the androgenic receptor (AR), the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). For MPA and NET, for example, we have been able to demonstrate anti-proliferative effects on E2-stimulated proliferation in MCF7 cells (Figure 1), an effect which seems to be mediated via AR and/or GR, may be in cross-talk with PR-mediated mechanisms [34]. Other progestogens may have agonistic or antagonistic effects via AR and GR depending on the hormonal milieu [34]. The anti-proliferative progestogen effect contrasts with the clinical data which clearly show an increased BC risk if synthetic progestogens to E replacement therapy is added, as described above for the WHI study [1], [2], [23]. Thus, our and other research is focusing on other mechanisms, mainly non-genomic mechanisms via growth factor effects and/or other receptors like membrane-bound steroid receptors.

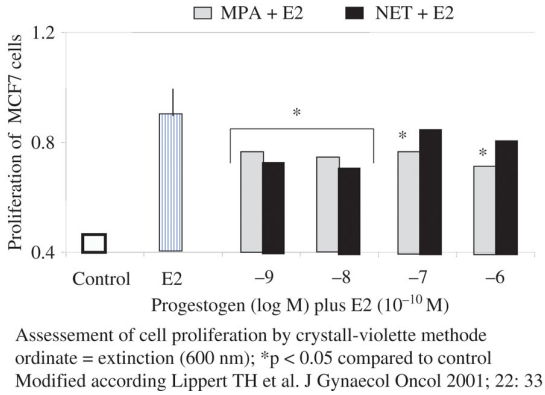

Figure 1: Progestogens can inhibit E2-mediated proliferation of BC cells (in contrast to clinical studies showing increased BC risk).

In addition to its classical activity in the nucleus, PR has been shown to interact non-classically with cytokine and growth factor signaling pathways at multiple levels to influence signaling cascades that play important roles in mammary cell proliferation and differentiation. This could be suggested, for example, within an in vitro experiment in T47D cells expressing only PR-B: epidermal growth factor (EGF) and progesterone act synergistically on promoters that drive the cell-growth regulatory genes *c-fos* and *p21*, neither of which contains a progesterone responsive element (PRE) [35].

Action of progestogens in the normal human breast

The natural progestogen, progesterone, plays a major role in the formation of lobular-alveolar structures during pregnancy [36]. Progesterone acts proliferatively via the direct regulation of cell cycle genes, synthesis of growth factors and of growth factor receptors. Through its role in lactation, progesterone also exerts a differentiating effect on the breast.

In vitro studies of the involvement of progesterone in breast epithelial proliferation have produced inconsistent results. In terms of HRT the experiments in normal human breast tissue may reflect the primary BC risk issue, i.e. the possible development of BC during use of HRT. It must be stressed, that in vitro and in vivo studies in cell cultures may point to mechanisms but never can replace clinical studies. In this context the results are important which could suggest that there may be differences between the various progestogens used in HRT.

Whereas estrogen clearly increases proliferation of normal breast epithelium, the effects of progesterone were inconsistent either alone or when combined with estrogen [37], mostly decreasing or having no ("neutral") effect on the proliferation of normal breast epithelium, e.g. explanted into nude mice [38], [39].

In our own experiments we compared the effects of the six synthetic progestogens (mostly used in HRT and/or hormonal contraception) as well as progesterone on the proliferation and apoptosis in the estrogen receptor-negative benign cell line *MCF-10A* [40]. Additionally, we included the most important stromal growth factors to investigate a possible influence of the stroma, whose influence on cells is often neglected in in vitro

experiments. The combination of the stroma-derived growth factors epithelial growth factor (EGF), basic-fibroblastic growth factor (FGF) and insulin-like growth factor-I (IGF-I) acted proliferatively compared to controls in our experiments. These growth factors were chosen as they are highly effective with regard to breast epithelial cell proliferation. The ratio of apoptosis to proliferation was significantly reduced when combining MPA or chlormadinone acetate (CMA) with the growth factor mixture. MPA (100 nM and 1 μM) reduced the ratio four-fold in comparison to growth factors alone. CMA only had a significant effect at 1 μM, reducing the ratio three-fold. Progesterone, norethindrone (NET), levonorgestrel (LNG), dienogest (DNG), gestodene (GSD) and 3-keto-desogestrel (KDG) had no significant effect on the growth factor-induced the stimulation of *MCF-10A*.

In summary, these results point to a difference within the progestogen class in terms of inducing or inhibiting the growth of benign human breast epithelial cells, dependent or independent of the effects of stromal growth factors. Thus, experimental data indicate that there is no "group effect", and the choice of progestogen may be important when considering a possible BC risk during hormone therapy.

Action of progestogens in human BC cells

During the very early years of HRT use it was generally believed that progestogen addition to estrogen could reduce the BC risk similar to the reduction of endometrial cancer risk. This was attributed to numerous in vitro and in vivo data, showing that progestogen addition can reduce the proliferation of BC cells (e.g. [41], [42], [43]). We have investigated the effect of MPA when added sequentially or continuously to estradiol in MCF-7 cells and observed that addition of MPA from 10 to 10 M upwards in the continuous combined model led to the inhibition of estradiol-induced growth [44] (Figure 1). Using the sequential combined model resulted in a greater inhibition at higher (10–8 to 10–6 M MPA) compared to lower concentrations (10–11 to 10–9 M MPA).

In another of our studies [40] we compared progesterone and seven synthetic progestogens in the same experimental model. Combining progestogens with E2 in the estrogen-receptor positive (ER+) HCC1500 cells showed that the progestogens CMA, MPA, NET, LNG, DNG, GSD and progesterone significantly increased the ratio of apoptosis to proliferation with differences within the progestogen class compared to E2 alone. MPA had the greatest effect, followed by NET. All progestogens except KDG inhibited the E2-induced effect. Combining progestogens with a combination of growth factors (EGF, FGF and IGF-I) and E2 on HCC1500 cells indicated that MPA, GSD, CMA and NET slightly reduced the proliferative effect of growth factors and E2. Progesterone, LNG, DNG and KDG elicited no significant effect. In conclusion, also in combination with stromal growth factors, certainly important for clinical effects, we did not find group effects, but our results indicate that certain different progestogens are able to inhibit the growth of malignant human breast epithelial cells independent of the effects of growth factors and E2.

Different mechanism(s) have been suggested for the progestogen-mediated antiproliferative effects in human BC cells. E initiates mitogenesis by inducing the transcription of important immediate early genes [45]. Using PI3K and MAP kinase inhibitors, it could be shown that cytoplasmic signaling pathways play an important role in the control of subsequent events in the cell cycle. E2 may also stimulate cell proliferation either through rapid, non-genomic effects, which involve the activation of MAP kinase, or by increasing growth factor production and consequently MAP kinase activity. Both mechanisms appear to be also involved in the antiproliferative action of progestogens and androgens [46]. In addition, progesterone and synthetic progestogens can enhance E2 dependent BC cell migration and invasion [47]. MPA was the most effective and drospirenone (DRSP) the least. However, these mechanisms appear to be cell-specific and no clear picture is evident.

Non-genomic actions of progestogens

Importance and actions mediated via PGRMC1

The classical genomic mechanism of PR action involves binding of progestogen to its receptors which are located in the cytoplasm. However, PRs can regulate gene expression using several distinct mechanisms which do not include binding of a receptor-ligand complex directly to DNA and therefore are known as non-genomic actions. These actions are believed to be mediated through membrane associated PRs which are associated with frequently activated various protein-kinase cascades. They happen so quickly that they cannot depend on the transcription of RNA and protein synthesis.

Binding sites localized at the cell membrane have been reported for several steroid hormones. According to our research one membrane protein binding progesterone receptor has been identified which may have importance in the development of BC in patients using HRT, the progesterone receptor membrane component 1, thereafter named PGRMC1.

PGRMC1 is a small protein of 22–26 kDa, which has a short extracellular domain, a transmembrane domain and a cytoplasm domain, the main part of the cytochrome P450 b5/heme binding domains, belonging to membrane-associated progesterone receptor proteins (MAPR) family, a member of multiple protein progesterone binding compounds [48]. PGRMC1 was found to be associated with membrane related progesterone receptor activities, expressed in many tissues, but showing different function. PGRMC1 is expressed in some solid tumors, but especially is over-expressed in BC tissue whereas it is not or only moderately expressed in normal breast epithelial tissue [49], [50], [51].

We recently analyzed the expression of PGRMC1 by immuno-histochemical staining of primary tumor tissues obtained from 69 BC participants [52]. We could demonstrate that overexpression of PGRMC1 is significantly correlated with larger tumor size and lymph node metastasis. High expression of PGRMC1 in the tumors assessed from BC patients had poorer disease-free and overall survival than BC participants with PGRMC1-low tumors. Multivariate analysis showed that PGRMC1 was an independent prognostic factor for BC. From these results we conclude that the expression of PGRMC1 might be useful for predicting prognosis in patients with BC.

Moreover, according several of own experiments in vitro and in animal studies [53], [54], [55], [56], [57], [58], [59], [60], [61] we suggest, that PGRMC1 expression in breast tissue also can predict if a certain progestogen, added to estrogen may increase the risk of BC, and one of the Editorials which was published discussing our results suggest, that the result in the WHI (increased BC risk by adding MPA to E) may be explained by our research on this issue [62].

For example, we found that progesterone conjugated with BSA-FITC (i.e. not membrane-permeable) was able to increase the proliferation of MCF-7/PGRMC1-cells independent of the classical progesterone receptor clearly suggesting non-genomic effects, because unconjugated progesterone did not show any effect [53]. In further investigations, we examined different synthetic progestogens on MCF-7/PGRMC1-cells in comparison to empty vector-controls. The results indicate that PGRMC1 mediates a progestogen-dependent proliferative signal in these cells, because no effect was found in the control cells. Testosterone-related progestogens (DNG, DRSP, DYD and NET) acted proliferatively, whereas progesterone and progesterone-related progestogens (CMA and NOM) were neutral with the exception of MPA and dydrogesterone in high concentrations. The reason for this discrepancy is not yet known.

We recently also compared the natural progestogen, progesterone, with norethisterone (NET), using an xenograft model. MCF7 cells and in a second model, also T47D cells, transfected with the PGRMC1 plasmid or empty vector, were injected into nude mice and E2 pellets were implanted. E2 plus NET increased the growth of the tumor overexpressing PGRMC1, but there was no change with progesterone. BC tissue transfected with empty vectors did not respond to either progestogen [61]. Thus, to our the best of knowledge for the first time also shown in in vivo research, PGRMC1 is important in terms of mediating proliferative effects of synthetic progestogens but not of the natural progesterone.

It is unclear how certain progestogens promote cell proliferation mediated by PGRMC1, also the mechanism of possible involvement in tumor formation predicting the prognosis of breast cancer patients is still not clear. PGRMC1 is involved in mediating protein kinase-related signals, it increased Akt and IkB phosphorylation and thus activated NFkB. Protein kinase PDK1 can make Akt phosphorylate. There is a PDK1 binding region in PGRMC1. However, the mechanism of PGRMC1-mediated Akt activation is unclear. The tyrosine T177 of the Erk2 in PGRMC1 has been shown phosphorylated in vivo. Western blot analysis showed that the basic Erk1/2 protein expression reduced by 40% in MCF-7/PGRMC1 cells compared with MCF-7 cells. Members of our working group recently investigated the activation mechanism of the receptor upon progestogen signals [63]. Phosphorylation of the receptor upon progestogen-treatment was investigated by mass spectrometry and Western blot analysis. Treatment of MCF7/PGRMC1 cells with NET induced phosphorylation of the receptor at the casein kinase2 (CK2) phosphorylation site Ser181, which can be decreased with CK2 inhibitor quinalizarin. Further, point mutation of the Ser181 phosphorylation site in MCF7/PGRMC1 cells impaired proliferation upon NET treatment. Based on these and other experiments we suggest that phosphorylation of the CK2 binding site is essential for the activation of the receptor upon progestogen binding.

In summary, evaluation of the expression of PGRMC1 could identify, especially women at risk, and could predict which progestogens should not been used in the individual patient with the need of HRT. In addition, as we recently published [64], the assessment of PGRMC1 may also predict the risk using other menopausal regimens like herbal substances. This could become routine if we are successful in also evaluating this marker in the blood correlating with the BC incidence in clinical endpoint studies – a main topic of our present research.

Clinical studies investigating BC risk during HRT

As already outlined in the introduction, regarding clinical studies on the risk of BC using E/progestogen therapy, the only placebo-controlled trial has been the WHI, showing that addition of MPA to CEE does increase the BC risk, although this increase in the first evaluation of the interventional phase was not significant (1.26; 95% CI 1.00–1.59) [1], was only significant in the second evaluation (1.24; 95% CI 1.01–1.54) [65], and the recent 18-year cumulative follow-up evaluation did show an non-significant increase of mortality (1.44; 95% CI 0.97–2.15) [23]. However, striking is the clear difference to CEE-only therapy [2], the decrease of risk during the interventional phase being almost significant (0.77; 95% CI 0.59–1.01), and the decrease of mortality in the 18-year cumulative follow-up evaluation has been significant (0.55; 95% CI 0.33–0.92) [23]. Thus, the possible negative impact of the progestogen component has also been well demonstrated in the clinical studies which is supported by the described experimental evidence. The most important studies investigating the risk of BC for combined HRT are listed in Table 2 showing the duration of the treatment, relative risks (with 95% CI), and (if applicable) also the difference between sequential and continuous combined HRT. Most of these studies have been already described elsewhere (e.g. in this journal [21], [22]).

Table 2: Observational studies on breast cancer risk during combined HRT using MPA, NETA or other progestogens.

First author, year		Relative risk or odds ratio (95% CI)				
	Duration	Estrogen/Progestin	Sequential	Continuous	MPA	Others
Magnusson, 1999	Ever	1.63 (1.37–1.94)	1.48 (1.08–2.04)	1.41 (1.09–1.83)	–	+
Persson, 1999	1–6 years	1.4 (0.9–2.3)			–	+
	>6 years	1.7 (1.1–2.6)				
Schairer, 2000	<4 years		1.1 (0.8–1.7)		+	–
	>4 years		1.5 (1.0–2.4)			
Ross, 2000	5 years	1.24 (1.07–1.45)	1.38 (1.13–2.68)	1.09 (0.88–1.30)	+	–
Chen, 2002	>5 years	1.49 (1.29–1.74)			+	–
Newcomb, 2002	>5 years	1.58 (1.16–2.15)			+	–
Weiss, 2002	>5 years	1.37 (1.06–1.77)	1.00 (0.69–1.46)	1.54 (1.10–2.17)	+	–
Porch, 2002	<5 years	1.11 (0.81–1.52)			+	–
	>5 years	1.76 (1.29–2.39)				
De Lignieres, 2002	>5 years			Progesterone 0.98 (0.65–1.5)	–	+
HERS, 2002	6.8 years	1.27 (0.84–1.94)			+	–
WHI, 2002	5.2 years	1.26 (1.00–1.59)			+	–
WHI, 2003	5.6 years	1.24 (1.01–1.54)			+	–
Li, 2003	>15 years	2.0 (1.3–3.3)	2.9 (1.3–6.6)	1.8 (1.0–3.3)	+	–
MWS, 2003	Current user	2.0 (1.88–2.12)			–	+
Olsson, 2003	>4 years		2.23 (0.90–5.56)	4.60 (2.38–8.84)	–	+
Jernström, 2003	5 years			3.3 (1.9–5.6)	–	+
Stahlberg, 2004	6 years	2.7 (1.96–3.73)			–	+
Opartny, 2008	6 years	Oral: 1.38 (1.27–1.49) Transdermal Combi-Patch 1.08 (0.81–1.43)	1.35 (1.21–1.46)	1.29 (1.07–1.56)		
Lyytinen, 2009	<5 years	1.31 (1.20–1.42)	1.78 (1.64–1.90)	2.44 (2.17–2.72)	+	+
Fournier, 2008	8.1 years	Synthetic progestins 1.69 (1.50–1.91) Progesterone 1.0 (0.83–1.22) Dydrogesterone 1.16 (0.94–1.43)			–	+
Fournier, 2014	78,353 women 881,290 person-years of postrnen opausal follow-up	progesterone or dydrogesterone 1.22 (1.11–1.35) Other progestins 1.87 (1.71–2.04)				

Cordina, 2013	>4 Jahre	Progesterone 0.79 (0.37–1.71) Synthetic progestins 2.07 (1.26–3.39)	2.00 (1.18–3.41)	2.70 (0.69–12.2)	+	+
Jones, 2016	5.4 Jahre >15 Jahre	2.74 (2.05–3.65) 3.27 (1.53–6.99)			+	+

All studies including references up to 2010 are reviewed in Mueck, [10]

It should be mentioned that from the most recent UK Breakthrough Generations Study (BGS) [66] it was suggested that the true risk of using combined HRT is higher as has been observed in clinical trials (including the WHI). However, there have been some critiques on this study, e.g. the small numbers of cases and no update of HRT-use after recruitment. In this cohort, 39,183 women with known age of menopause were included, who used E-only (CEE or E2) already for 6.6 years (mean) (2.5–10.5 years) and combined HRT for 5.5 (2.5–9.5) years (progestogens MPA, norgestrel, NETA). During the 6 years of follow-up 775 breast cancers were diagnosed. For current users of combined HRT an increased risk was found (2.74; 95% CI 2.05–3.65; mean duration 5.4 years), whereas with E-only even after use of 15 years the risk was not increased (1.14; 95% CI 0.42–3.08).

In most studies investigating combined HRT the synthetic progestogens MPA or NET/NETA have been used (Table 2). However, at least five observational studies support our and other experimental data as described already, suggesting a lower risk using progesterone or dydrogesterone at least up to 5–8 years compared to synthetic progestogens [67] which also has been an official statement of societies like the International Menopause Society (IMS) [3]. Three of these five studies are listed within Table 2 [68], [69], [70]. In addition, regarding the use of dydrogesterone within two other studies [71], [72] the data have been analyzed by calculation of the BC incidence rates. [Standardized incidence ratios (SIR) were calculated by dividing the numbers of observed cases by the respective expected numbers based on age-standardized background population.] The Finnish Cohort Study [71] (n = 50,210, women > 50 years, HRT > 5 years) did not show an increased risk using dydrogesterone (SIR 1.13; 95% CI 0.49–2.22) in contrast to the use of other progestogens like MPA (SIR 1.64; 95% CI 1.49–1.79) and NETA (SIR 2.03; 95% CI 1.88–2.18). Likewise within a nested case-control study a lower risk (or even some protective action) during use of dydrogesterone was suggested using the UK-based General Practice Research Data base (GPRD) [72] analyzing 1482 incident cancer cases and 8892 matched control women: The incidence rate/1000 person-years using no HRT was 3.16 (95% CI 2.91–3.42), using dydrogesterone 2.41 (95% CI 1.81–3.15), whereas other combined HRT regimens increased the risk (3.28; 95% CI 3.01–3.55).

Detailed analyses of these observational studies using progesterone or its retro-isomer dydrogesterone suggest that the use of those progestogens longer than 5–8 years cannot exclude an increase of BC risk although the trend of increase is smaller compared with synthetic progestogens like NET or MPA. Most promising for the potential of a lower BC risk compared to synthetic progestogens is the use of the micronized progesterone, also comparing our own and other experimental data (as described in this review), which have also been summarized within an extensive review very recently [73].

In the context of the importance of the progestogen component increasing the risk of BC, of interest may be the question, whether the sequential use of HRT may confer a lower risk of BC compared to continuous combined use, which could be suggested by some, but not all studies listed in Table 2 which are investigating this issue. In addition, this also has been observed in the large European Prospective Investigation into Cancer and Nutrition trial [74] performed in 10 European countries (n = 133,744 postmenopausal women) with a mean duration of HRT use of 8.6 years (4312 primary BCs) – relative risk (RR) of continuous to sequential HRT was assessed as RR 1.43 (95% CI 1.19–1.72). Also in this large trial the risk of using E-only was significantly lower (although itcould not be excluded) compared to combined HRT – ratio combined HRT vs. E-only RR 1.77 (95% CI; 1.40–2.24). However, if the HRT-regimen (sequential vs. continuous combined) elicits different risks, still has to remain as an open question, considering also that for sequential use the dosage of the progestogen mostly must be higher compared to daily use to get enough endometrial protection.

BC mortality after use of HRT

The mortality due to BC after use of E-only as well as of combined HRT decreased about 30% in about a dozen observational studies [75]. Especially striking is the low risk of mortality observed within the most recent huge Finnish registry study on the basis of 489,105 women who were followed from the HRT initiation (3.3 million cumulative exposure years) to BC death [76]: Compared to the age-standardized background population the BC mortality risk was reduced in all HRT users with exposure for at most 5 years (standardized mortality ratio

0.56; 95% CI 0.52–0.60), more than 5 to 10 years (0.46; 95% CI 0.41–0.51), or more than 10 years (0.62; 95% CI 0.56–0.68). A significantly larger risk reduction was detected in the 50 to 59 years age group (0.33; 95% CI 0.29–0.37) compared with 60 to 69 (0.64; 95% CI 0.59–0.70) or 70 to 79 (0.78; 95% CI 0.69–0.87) years age groups.

The death risk reductions in E-only users tended to be larger in all age groups compared with combined HRT. This has also been observed in the recent 18-year follow-up of the WHI [23] – CEE alone vs. placebo HR 0.55 (95% CI 0.33–0.92) – whereas in the WHI using CEE+MPA the BC mortality was (although not significantly) increased (HR 1.44; 95% 0.97–2.15). As in the Finnish study only E, not CEE, has been used together with various progestogens (mostly not MPA), this difference might be explained by different types of HRT, but conclusive explanation for the difference needs more studies.

Conclusion

Regarding the difficult issue of "BC and HRT" and the importance of the choice of progestogen using combined HRT it can be summarized, in conclusion of experimental data and clinical studies, including the WHI:

1. The increased risk of breast cancer is primarily associated with the addition of a synthetic progestogen to E therapy and is related to duration of use. This has been shown in clinical studies and in our and other recent studies including experiments on non-genomic effects whereas older studies did suggest a decrease of risk adding progestogens to E. Experimental studies suggest increasing risk related to increased dosage of the progestogen component, but clinical studies regarding this question are very rare.
2. The risk may be lower with micronized progesterone or dydrogesterone than with a synthetic progestogen suggested from clinical and our and other experimental studies including the non-genomic effects.
3. Derived from clinical studies, the risk of E-only therapy is lower compared to combined HRT. However, the risk cannot be excluded, but perhaps is observed only during long-term use because proliferation is slow and carcinoprotective effects like apoptosis induced by Es can work. In certain populations E-only therapy even may be protective.
4. Derived from our own extensive research, especially women expressing membrane-bound receptors for progestogen action (e.g. progesterone receptor membrane component 1, PGRMC1) may be at increased risk for developing BC with aggressive phenotype and poor prognosis. Thus, in future, screening for these receptors could be very valuable to identify women who would be at increased risk using HRT and to predict the prognosis of breast cancer. For clinical practice this may be more useful than performing more studies because despite more than 70 studies there are still unanswered questions.

From existing evidence it can be concluded that the overall risk is small (about 0.1–0.5%/year) and mortality due to BC for patients using HRT may even be decreased. Since another randomized placebo-controlled study with the design like the WHI, but using other HRT regimens in younger populations, may never be performed, rather than performing more and more controversial observational studies, the definitive solution of the problem for the best choice of the progestogen could be to screen for those few patients who are at risk and evaluate risk factors and mechanisms for BC development.

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References

[1] Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. J Am Med Assoc. 2002;288:321–33.

[2] Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, et al. Women's Health Initiative Steering Committee. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. J Am Med Assoc. 2004;291:701–12.

[3] Baber RJ, Panay N, Fenton A, (on behalf of the IMS Writing Group). 2016 IMS Recommendations on women's midlife health and menopause hormone therapy. Climacteric. 2016;19:109–50.

[4] Neves-E-Castro M, Birkhauser M, Samsioe G, Lambrinoudaki I, Palacios S, Borrego RS, et al. EMAS position statement: the ten point guide to the integral management of menopausal health. Maturitas. 2015;81:88–92.

[5] North American Menopause Society. The 2012 hormone therapy position statement of: the North American Menopause Society. Menopause. 2012;19:257–71.

[6] German S3 Guideline on HRT (Ortmann O, on behalf of 19 German Societies). Hormonal therapy in peri- and postmenopause. Short version of interdisciplinary S3 guideline. Frauenarzt. 2009;10:840–51.

[7] Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, et al. Postmenopausal hormone therapy: an Endocrine Society scientific statement. J Clin Endocrinol Metab. 2010;95(Suppl. 1):s1–66.

[8] NICE Guidelines. Menopause: diagnosis and management. Available from: https://www.nice.org.uk/guidance/ng23/resources/menopause-diagnosis-and-managment-1837330217413. Accessed: 12 Dec 2015.

[9] de Villiers T, Hall JE, Pinkerton J, Cerdas Perez S, Reese M, Yang C, et al. Revised global consensus statement on menopausal hormone therapy. Climacteric. 2016;19:313–5.

[10] US Preventive Services Task Force, Grossman DC, Curry SJ, Owens DK, Barry MJ, Davidson KW, et al. Hormone therapy for the primary prevention of chronic conditions in postmenopausal women: US Preventive Services Task Force Recommendation Statement. J Am Med Assoc. 2017;318:2224–33.

[11] Stefanick ML, Anderson GL, Margolis KL, Hendrix SL, Rodabough RJ, Paskett ED, et al. Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. J Am Med Assoc. 2006;295:1647–57.

[12] Scarabin PY, Oger E, Plu-Bureau G (for the ESTHER Study Group). Differential association of oral and transdermal oestrogen replacement therapy with venous thromboembolism risk. Lancet. 2003;362:428–32.

[13] Canonico M, Plu-Bureau G, Lowe GD, Scarabin P-Y. Hormone replacement therapy and risk of venous thromboembolism in postmenopausal women: a systematic review and meta-analysis. Br Med J. 2008;336:1227–31.

[14] Renoux C, Dell-Aniello S, Suissa S. Hormone replacement therapy and the risk of venous thromboembolism: a population-based study. J Thrombosis Haemostasis. 2010;8:979–86.

[15] Simon JA, Laliberté F, Duh MS, Pilon D, Kahler KH, Nyirady J, et al. Venous thromboembolism and cardiovascular disease complications in menopausal women using transdermal versus oral estrogen therapy. Menopause. 2016;23:600–10.

[16] Renoux C, Dell'Aniello S, Garbe D, Suissa S. Transdermal and oral hormone replacement therapy and the risk of stroke: a nested case-control study. Br Med J. 2010;340:c2519.

[17] Speroff L. Transdermal hormone therapy and the risk of stroke and venous thrombosis. Climacteric. 2010;13:429–32.

[18] Canonico M, Carcaillon L, Plu-Bureau G, Oger E, Singh-Manoux A, Tubert-Bitter P, et al. Postmenopausal hormone therapy and risk of stroke. Impact of the route of estrogen administration and type of progestogen. Stroke. 2016;47:1734–41.

[19] Canonico M, Scarabin P-Y. Oral versus transdermal estrogens and venous thromboembolism in postmenopausal women: what is new since 2003? Menopause. 2016;23:587–8.

[20] Mueck AO. Postmenopausal hormone replacement therapy and cardiovascular disease: the value of transdermal estradiol and micronized progesterone. Climacteric. 2012;15(Suppl 1):11–7.

[21] Mueck AO, Seeger H. Shapiro S. Risk of breast cancer during hormone replacement therapy: Mechanisms. Horm Mol Biol Clin Invest. 2010;3:329–39.

[22] Mueck AO, Ruan X. Benefits and risks during HRT – main safety issue breast cancer. Horm Mol Biol Clin Invest. 2011;5:105–16.

[23] Manson JE, Aragaki AK, Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, et al. Menopausal Hormone Therapy and long-term all-cause and cause-specific mortality: the women's health initiative randomized trials. J Am Med Assoc. 2017;318:927–38.

[24] Manson JE, Kaunitz AM. Menopause management – Getting clinical care back on track. N Engl J Med. 2016;374:803–6.

[25] Langer RD. The evidence base for HRT: what can we believe? Climacteric. 2017;20:91–6.

[26] Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS, Horwitz KB. A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol Endocrinol. 1994;8:1347–60.

[27] Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. Science. 2000;289:1751–4.

[28] Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci USA. 2003;100:9744–9.

[29] Lange CA, Richer JK, Horwitz KB. Hypothesis: progesterone primes breast cancer cells for cross-talk with proliferative or antiproliferative signals. Mol Endocrinol. 1999;13:829–36.

[30] Bergink EW, van Meel F, Turpijn EW, van der Vies J. Binding of progestagens to receptor proteins in MCF-7 cells. J Steroid Biochem. 1983;19:1563–70.

[31] Kontula K, Janne O, Vihko R, de Jager E, de Visser J, Zeelen F. Progesteronebinding proteins: In vitro binding and biological activity of different steroidal ligands. Acta Endocrinol. 1975;78:574–92.

[32] Ghatge R, Jacobsen B, Schittone S, Horwitz K. The progestational and androgenic properties of medroxyprogesterone acetate: gene regulatory overlap with dihydrotestosterone in breast cancer cells. Breast Cancer Res. 2005;7:R1036–50.
[33] Bray JD, Jelinsky S, Ghatge R, Bray JA, Tunkey C, Saraf K, et al. Quantitative analysis of gene regulation by seven clinically relevant progestins suggests a highly similar mechanism of action through progesterone receptors in T47D breast cancer cells. J Steroid Biochem Mol Biol. 2005;97:328–41.
[34] Moore NL, Hickey TE, Butler LM, Tilley WD. Multiple nuclear receptor signalling pathways mediate the actions of synthetic progestins in target cells. Mol Cell Endocrinol. 2012;397:60–70.
[35] Jacobsen BM, Schittone SA, Richer JK, Horwitz KB. Progesterone-independent effects of human progesterone receptors (PRs) in estrogen receptor-positive breast cancer: PR isoform-specific gene regulation and tumor biology. Mol Endocrinol. 2005;19:574–87.
[36] Topper YJ, Freeman CS. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol Rev. 1980;60:1049–106.
[37] Clarke CL, Sutherland RL. Progestin regulation of cellular proliferation. Endocr Rev. 1990;11:266–301.
[38] McManus MJ, Welsch CW. The effect of estrogen, progesterone, thyroxine, and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. Cancer. 1984;54:1920–7.
[39] Laidlaw IJ, Clarke RB, Howell A, Owen AW, Potten CS, Anderson E. The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone. Endocrinology. 1995;136:164–71.
[40] Krämer EA, Seeger H, Krämer B, Wallwiener D, Mueck AO. The effect of progesterone, testosterone and synthetic progestogens on growth factor- and estradiol-treated human cancerous and benign breast cells. Eur J Obstet Gynecol Reprod. 2006;27:39–141.
[41] van der Burg B, Kalkhoven E, Isbrücker L, de Laat SW. Effects of progestins on the proliferation of estrogen-dependent human breast cancer cells under growth factor-defined conditions. J Steroid Biochem Mol Biol. 1992;42:457–65.
[42] Schoonen WG, Joosten JW, Kloosterboer HJ. Effects of two classes of progestins, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: 1.MCF-7 cell lines. J Steroid Biochem Mol Biol. 1995;55:423–37.
[43] Cappelletti V, Miodini P, Fioravanti L, DiFronzo G. Effect of progestin treatment on estradiol- and growth factor-stimulated breast cancer cell lines. Anticancer Res. 1995;15:2551–6.
[44] Lippert C, Seeger H, Wallwiener D, Mueck AO. The effect of medroxyprogesterone acetate and norethisterone on the estradiol stimulated proliferation in MCF-7 cells: comparison of continuous combined versus sequential combined estradiol/progestin treatment. Eur J Gynaecol Oncol. 2001;22:331–5.
[45] Lobenhofer EK, Huper G, Iglehart JD, Marks JR. Inhibition of mitogen-activated protein kinase and phosphatidylinositol 3-kinase activity in MCF-7 cells prevents estrogen-induced mitogenesis. Cell Growth Differ. 2000;11:99–110.
[46] Santen RJ, Xinde Song R, McPherson R, Kumar R, Adam L, Jeng MH, et al. The role of mitogen-activated protein (MAP) kinase in breast cancer. J Steroid Biochem Mol Biol. 2002;80:239–56.
[47] Fu XD, Giretti MS, Goglia L, Flamini MI, Sanchez AM, Baldacci C, et al. Comparative actions of progesterone, medroxyprogesterone acetate, drospirenone and nestorone on breast cancer cell migration and invasion. BMC Cancer. 2008;8:166–71.
[48] Losel RM, Besong D, Peluso JJ, Wehling M. Progesterone receptor membrane component 1 – many tasks for a versatile protein. Steroids 2008;73:929–34.
[49] Cahill MA. Progesterone receptor membrane component 1: an integrative review. J Steroid Biochem Mol Biol. 2007;105:16–36.
[50] Crudden G, Loesel R, Craven RJ. Overexpression of the cytochrome p450 activator hpr6 (heme-1 domain protein/human progesterone receptor) in tumors. Tumour Biol. 2005;26:142–6.
[51] Neubauer H, Ma Q, Zhou J, Yu Q, Ruan X, Seeger H, et al. Possible role of PGRMC1 in breast cancer development. Climacteric 2013;16:509–13.
[52] Ruan X, Zhang Y, Mueck AO, Willibald M, Seeger H, Fehm T, et al. Increased expression of progesterone membrane component 1 is associated with aggressive phenotype and poor prognosis in ER-positive and – negative breast cancer. Menopause. 2017;24:203–9.
[53] Neubauer H, Adam G, Fehm T, Seeger H, Neubauer H, Solomayer E, et al. Membrane-initiated effects of progesterone on proliferation and activation of VEGF gene expression in human breast cancer cells. Climacteric. 2009;12:230–9.
[54] Neubauer H, Yang Y, Seeger H, Fehm T, Tong X, Cahill MA, et al. The presence of a membrane-bound progesterone receptor sensitizes the estradiol-induced effect on the proliferation of human breast cancer cells. Menopause. 2011;18:845–50.
[55] Ruan X, Neubauer H, Yang Y, Schneck E, Schultz S, Fehm T, et al. Progestogens and membrane-initiated effects on the proliferation of human breast cancer cells. Climacteric. 2012;15:467–72.
[56] Ruan X, Neubauer H, Schneck H, Schultz S, Fehm T, CaHill MA, et al. Nomegestrol acetate sequentially or continuously combined to estradiol did not negatively affect membrane-receptor associated progestogenic effects in human breast cancer cells. Gynecol Endocrinol. 2012;28:863–6.
[57] Schneck H, Ruan X, Seeger H, Cahill MA, Fehm T, Neubauer H, et al. Membrane-receptor initiated proliferative effects of dienogest in human breast cancer cells. Gynecol Endocrinol. 2013;29:160–3.
[58] Neubauer H, Ruan X, Schneck H, Seeger H, Cahill MA, Liang Y, et al. Overexpression of progesterone receptor membrane component 1: possible mechanism for increased breast cancer risk with norethisterone in hormone therapy. Menopause. 2013;20:504–10.
[59] Zhou J, Yu Q, Chen R, Seeger H, Fehm T, Cahill MA, et al. Medroxyprogesterone acetate- driven increase in breast cancer risk might be mediated via cross-talk with growth factors in the presence of progesterone receptor membrane component-1. Maturitas. 2013;76:129–33.
[60] Zhang Y, Ruan X, Willibald M, Seeger H, Fehm T, Neubauer H, et al. May progesterone receptor membrane component 1 (PGRMC1) predict the risk of breast cancer? Gynecol Endocrinol. 2016;32:58–60.
[61] Zhao Y, Ruan X, Wanga H, Lia X, Gua M, Wang L, Li Y, et al. The presence of membrane-bound progesterone receptor induces growth of breast cancer with norethisterone but not with progesterone: a xenograft model. Maturitas. 2017;102:26–33.
[62] Stanczyk FZ. Editorial. Can the increase in breast cancer observed in the estrogen plus progestin arm of the Women's Health Initiative trial be explained by progesterone receptor membrane component 1? Menopause. 2011;18:833–4.

[63] Willibald M, Bayer G, Stahlhut V, Poschmann G, Stühler K, Gierke B, et al. Progesterone Receptor Membrane Component 1 is Phosphorylated upon Progestin Treatment in Breast Cancer Cells. Oncotarget (advanced publications). Available from: www.impactjournals.com/oncotarget/. Accepted: 28 Jun 2017.
[64] Seeger H, Ruan X, Neubauer H, Brucker S, Mueck A. Membrane-initiated effects of Serelys® on proliferation and apoptosis of human breast cancer cells. Gynecol Endocrinol. 2017; epub ahead (Nov 5, 2017).
[65] Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. J Am Med Assoc. 2003;289:3243–53.
[66] Jones ME, Schoemaker MJ, Wright L, McFadden E, Griffin J, Thomas D, et al. Menopausal hormone therapy and breast cancer: what is the true size of the increased risk? Br J Cancer. 2016;115:607–15.
[67] Asi N, Mohammed K, Haydour Q, Gionfriddo MR, Vargas OL, Prokop LJ, et al. Progesterone vs. synthetic progestins and the risk of breast cancer: a systematic review and meta-analysis. Systematic Rev. 2016;5:121–9.
[68] de Lignières B, de Vathaire F, Fournier S, Urbinelli R, Allaert F, Le MG, et al. Combined hormone replacement therapy and risk of breast cancer in a French cohort study of 3175 women. Climacteric. 2002;5:332–40.
[69] Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone therapies: results from the E3N cohort study. Breast Cancer Res Treat. 2008;107:103–11.
[70] Cordina-Duverger E, Truong T, Anger A, Sanchez M, Arveux P, Kerbrat P, et al. Risk of breast cancer by type of menopausal hormone therapy: a case-control study among post-menopausal women in France. PLoS One. 2013;11:1–9.
[71] Lyytinen H, Pukkala E, Ylikorkala O. Breast cancer risk in postmenopausal women using estradiol-progestogen therapy. Obstet Gynecol. 2009;113:65–73.
[72] Schneider C, Jick SS, Meier CR. Risk of gynecological cancers in users of estradiol/dydrogesterone or other HRT preparations. Climacteric. 2009;12:514–24.
[73] Stute P, Wildt L, Neulen J. The impact of micronized progesterone on breast cancer risk: a systematic review. Climacteric. 2018;21:111–22.
[74] Bakken K, Fournier A, Lund E, Waaseth M, Dumeaux V, Clavel-Chapelon F, et al. Menopausal hormone therapy and breast cancer risk: impact of different treatments. The European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2011;128:144–56.
[75] Nanda K, Bastian LA, Schulz K. Hormone replacement therapy and the risk of death from breast cancer: a systematic review. Am J Obstet Gynecol. 2002;186:325–34.
[76] Mikkola TS, Savolainen-Peltonen H, Tuomikoski P, Hoti F, Vattulainen P, Gissler M, et al. Reduced risk of breast cancer mortality in women using postmenopausal hormone therapy: a Finnish nationwide comparative study. Menopause. 2016;23:1199–203.